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Previews

# Stem Cells and the Rate of Living

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**The "rate-of-living theory" is an ancient explanation of longevity which holds that aging occurs due to the exhaustion of some finite substance—breaths, heartbeats, etc. While this theory as originally conceived has been debunked, new work (Ruzankina et al. [2007], in this issue of *Cell Stem Cell*) suggests that mammals in fact do have a finite number of stem cell replications per life.**

Most mammalian organs are in part composed of cells that have a finite lifespan but which are constantly replenished from a source of stem and other self-renewing compartments. As we grow old, our stem cells appear to lose replicative capacity, and this in turn causes some aspects of aging (reviewed in Sharpless and DePinho [2004]), but it is not precisely known what causes this decline in regenerative potential. Provocative hints have come from the observation that humans and mice with congenital deficiencies of DNA repair and metabolism exhibit premature stem cell dysfunction (Prasher et al., 2005) and features of accelerated aging (de Boer et al., 2002; Vogel et al., 1999). Likewise, exposure to DNA-damaging agents such as chemo- and radiotherapy induces some aspects of aging also in the setting of a durable degradation of stem cell function (Knudsen et al., 1999; Testa et al., 1985; Wang et al., 2006). A problem from these lines of evidence, however, is that DNA damage affects all cells in these models, including rapidly proliferating progeny that lack self-renewal capacity but which are crucial to organismal homeostasis. Depletion of these progeny, for example after a sublethal dose of radiotherapy, forces an immense replicative cost on the remaining stem cells, which have to divide in order to regenerate depleted progenitors. Therefore, one cannot tell from these observations whether the decline in stem cell function results from DNA-damaging events within stem cell compartments themselves or whether the stress of forced and repeated proliferation leads to exhaustion of self-renewing cells. While many of us would have guessed that intrinsic DNA damage is the lesion in old stem cells, an intriguing new study from Ruzankina et al. (2007) additionally suggests that replication itself comes with some cost to the self-renewing compartments.

The authors took a very clever approach to get at this question. Using a conditionally inactivatable allele, the authors excised the ataxia-telangectasia and Rad3-related (ATR) gene in a wide variety of adult murine tissues through transient somatic activation of a tamoxifen (TAM)-inducible Cre recombinase. ATR is a PI3K-like kinase that activates multiple downstream signaling networks in response to DNA damage, particularly DNA replication intermediates such as stalled replication forks. Prior work had demonstrated that ATR deficiency is highly toxic to replicating cells (Brown and Baltimore, 2000), and therefore it is not surprising that acute somatic ATR deletion induced rapid depletion of proliferating cells in tissues such as the bone marrow and intestine. Consistent with the experience of others, Cre-mediated excision was not 100% efficient, and TAM-treated mice quickly repopulated these proliferative tissues through the replication of ATR nonrecombined (i.e., functional) cells. Just as is the case after a large dose of sublethal radiation, for all intents, these reconstituted mice appeared normal by 1 month post-TAM. Gratifyingly, largely quiescent tissues (e.g., brain) in TAM-treated mice retained ATR-deficient cells, yet the animals developed little or no phenotypic effects of this, suggesting the principal functions of ATR are related to cell division.

While these observations are important for those interested in DNA repair and ATR, we believe the finding that will be of greatest general scientific interest is what happened next. While TAM-treated mice rapidly reconstituted missing proliferative cells, this episode of forced regeneration appears to come at some durable cost. By 3 months after treatment, the TAM-exposed mice developed a carefully detailed, widespread, and impressive progeroid phenotype including graying, osteopenia, changes in skin thickness, and loss of hematopoietic and lymphoid progenitors. Importantly, the authors show that these phenotypes occur in tissues in which virtually all the cells harbor the unrecombined (intact) *ATR* allele. This finding indicates that the accelerated aging occurs in ATR-competent tissues and therefore is the result of forced regeneration rather than ATR deficiency. The authors offer evidence that the obligate tissue regeneration leads to stem cell exhaustion in a few compartments, contributing to the aging phenotypes observed. While related results have been reported after regeneration induced by the use of noxious agents (e.g., IR, chemotherapy), the major strength of this genetic approach is that it illustrates that cellular depletion can act in an *extrinsic* manner to induce aging. That is, stem cell exhaustion can occur as a result of proliferative stress forced through normal homeostatic mechanisms.

There are several advantages to this experimental approach: the phenotype is seen in ATR competent cells,

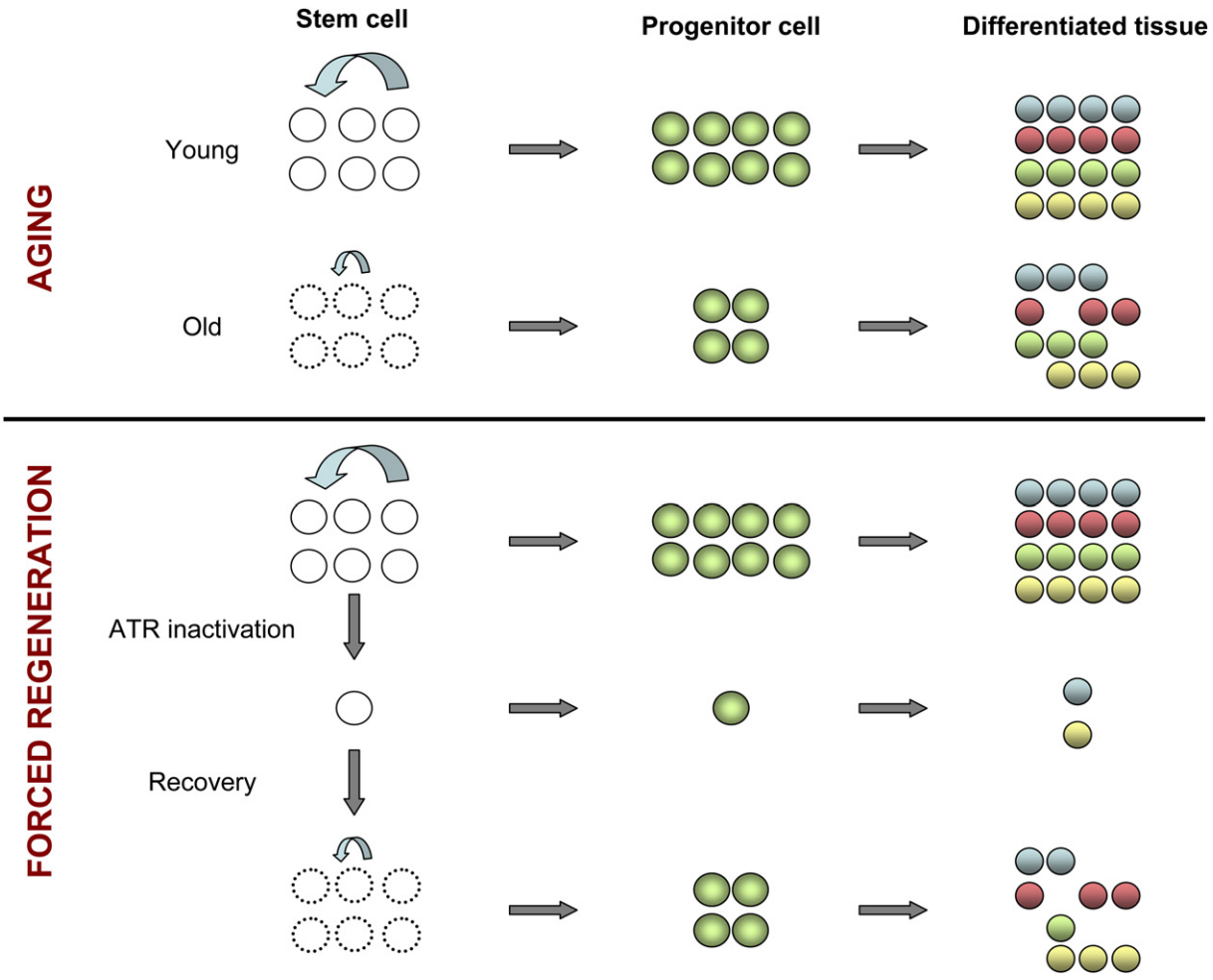


**Figure 1. A Model of the Effects of Physiologic Aging versus Acute ATR Inactivation on Long-Term Stem Cell Function**
Stem cells have the capacity to self renew (light blue arrow) and asymmetrically divide to produce progenitor cells, which in turn give rise to differentiated cellular components. With physiologic aging, stem cell quality, but not necessarily quantity, declines (shown with a hatched outline). Acute somatic ATR excision is cell-autonomously lethal to proliferating cells and is followed by tissue reconstitution as a result of the replication of ATR-competent stem cells. Enforced regeneration, however, accelerates aging in many tissues, presumably because of a "premature aging" of the stem cell compartments.

there is no issue of toxicity from Cre recombinase expression, there is no need for extrinsic DNA-damaging agents, etc. However, as the authors note, the results come with a significant experimental caveat: this study does not tell us in which ATR-competent cells the aging actually occurs. We presume the cost of forced regeneration is greatest in self-renewing compartments (e.g., Figure 1), but because the Cre allele is expressed ubiquitously, it is possible that forced regeneration could be toxic to other compartments as well, or even instead. For example, while we favor the model that somatic excision of ATR in the skin causes premature graying because forced regeneration exhausts melanocytic stem cells, this interpretation has not been explicitly shown. In fact, the stem cell "niche" in other systems has been demonstrated to play an important role in determining stem cell function with age (Conboy et al., 2005). Therefore, as the authors point out, it is possible that ATR excision and subsequent regeneration somehow damage the niche rather than the self-renewing cells within. Delineating the effects of forced regeneration on supporting cells such as those comprising the niche versus the stem cells themselves will be an important question for future study.

In summary, these new data (Ruzankina et al., 2007) suggest that maybe there is something to this rate-of-living hypothesis after all. While there clearly is no finite number of heart beats per human life span, there may be a limit to how many times a stem cell can divide. The molecular nature of the barrier that limits total number of stem cell proliferations (or "the replicometer," in Hayflickean parlance) is not known, but it is not likely to be telomere based in these experiments using inbred rodents harboring ample telomeric reserve (Sharpless and DePinho, 2004). An alternative model is that the barrier is stochastic in nature: perhaps there

is a small chance per stem cell division of a disaster of replication that leads to a dysfunctional, but not dead, stem cell. In this model, with time these damaged stem cells accumulate, and eventually with aging, tissue regeneration is compromised as a result of their reduced function. If correct, this model has implications for regenerative medicine-based approaches for aging therapy: if cell division itself is toxic to adult, tissue-specific stem cells, from where will we make new ones when we grow old?

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# MAPK-ing Out the Pathways in Lung Stem Cell Regulation

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**Methods to isolate and characterize stem cell populations from the lung are emerging, making it possible to begin to map out the pathways that are required for stem cell function in the adult lung. A new study by Ventura et al. (2007) points to a requirement for MAPK14 (also known as p38α) in regulation of lung stem or progenitor cell proliferation and differentiation.**

The lung is a complex solid tissue that has eluded stem cell biologists for many years. Lung injury modeling in rodents has suggested that adult lung stem or progenitor cells exist, yet there has been a paucity of lineage relationship analysis in the pulmonary system and little demonstration that lung cells exhibit stem cell properties (recently reviewed by Rawlins and Hogan [2006]). The limited number of molecular markers for lung cells and previous work assigning stem/progenitor cell function without detailed analyses of self-renewal or differentiation are key factors which have precluded an understanding of stem cell identity in the lung and knowledge of the pathways required for adult lung homeostasis.

We previously demonstrated that cells termed bronchioalveolar stem cells (BASCs) are capable of self-renewal and differentiation (Kim et al., 2005). BASCs reside in the distal lung and coexpress Scgb1a1 (aka CC10, CCSP, or CCA), a marker of the bronchiolar epithelial cells (Clara cells), and prosurfactant protein C (SP-C), a marker of the type II alveolar epithelial cells (AT2 cells). BASCs are isolated prospectively from fresh lung homogenates using the cell surface phenotype Sca1+ CD34+ CD31− CD45− and cultured to self-renew or differentiate into Clara cells, AT2 cells, and type I alveolar cells (lung epithelial cells that perform gas exchange). The molecular pathways that regulate BASC function in vivo and in vitro remain to be defined.

Ventura and colleagues have now reported the isolation of a putative stem or progenitor cell population from the adult lung and show that the fate of these cells is altered by MAPK14 deletion (Ventura et al., 2007). Mice bearing a conditional null allele of *Mapk14* were mated to mice with large subunit RNA polymerase II-driven Cre-ERT2 mice (*RERTn*$^{ERT}$). Despite Cre-mediated recombination in diverse tissues after systemic tamoxifen treatment, the most prominent phenotype was a severe disruption of pulmonary morphology with a marked increase in cycling lung cells, more numerous SP-C+ cells, and a decrease in E-cadherin-positive cells that were collectively interpreted as lung differentiation defects.